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Effects of Caspase 9 Gene Polymorphism in Patients with Prostate Cancer

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Abstract. *Background: Prostate cancer is one of the most common solid tumors and the second leading cause of the death due to malignancy in men. Caspase 9 (CASP9) is a member of the intrinsic pathway and plays a central role in the apoptosis. Patients and Methods: Genotyping of the CASP9 (rs1052576) polymorphism were performed using real-time polymerase chain reaction for blood samples of prostate cancer patients (n=69) and controls (n=76). Results: There were no significant differences between the groups in the frequency of CASP9 genotypes (χ^2=1.363; p=0.506). Patients with CASP9 (rs1052576) CT genotype were 12.8 fold higher in pathological stage of pT2a compared to any other stages of cancer (OR=0.078, 95% CI= 0.009-0.062; p=0.004). Also TT genotype carriers were 11.3 times lower in pathological stage of pT2a (OR=11.33, 95% CI=2.39-53.748; p=0.000). C allele carriers were 11.36 fold higher in pathological stage of pT2a compared to any other stages of cancer (OR=0.088, 95% CI=0.019-0.418; p=0.002). Conclusion: CASP9 (rs1052576) C allele was decreasing the risk for pathological stage of patients with prostate cancer and also CT genotype had positive impact on pathological stage of patients with prostate cancer. CASP9 (rs1052576) TT genotype was seemed to be associated with higher risk of pathological stage. Those results implicated that CASP9 variations could be associated with severity of prostate cancer.*

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Caspase protein family includes protease enzymes which are regulating programmed cell death. Caspase 9 (CASP9) is a member of the intrinsic pathway and plays a central role in the mitochondrial apoptotic pathway. The intrinsic pathway is initiated by the release of cytochrome *c* from mitochondria in response to cellular stress. After releasing of cytochrome *c* from mitochondria, it interacts with apoptotic protease activating factor 1 (Apaf-1), procaspase-9 and deoxyadenosine triphosphate (dATP) to form a multiprotein complex which called apoptosome. Apoptosome actives CASP9 which triggers a cascade of effector caspases. It was shown that CASP 9 was the direct target for regulatory phosphorylation by multiple protein kinases activated in response to extracellular growth/survival factors, osmotic stress or during mitosis. Apoptosis can be triggered by a variety of factors such as DNA damage or oxidative stress (1, 2).

Prostate cancer is the most common type of cancer and a leading cause of death among men. Because of slow progression and showing similar symptoms with bening prostatic hyperplasias, risk factor determination may be valuable for prostate cancer diagnosis. There is no clear cause or symptom known for prostate cancer, therefore genetic risk factors may help illuminate the diagnosis. Several studies have shown that having a first-degree relative with prostate cancer increases the risk of developing prostate cancer twice and genome-wide association studies (GWAS) had shown that some single nucleotide polymorphisms were increasing prostate cancer risk (2, 3).

Patients and Methods

Patient (n=69) and control (n=76) groups were selected during clinical examination at Yeditepe University, Urology Department, Turkey. Radiologic and pathological analysis performed in all patients for evaluating pathological and clinical information. After obtaining informed consent, and peripheral blood samples collected by EDTA containing tubes. Demographic characteristics (age, body mass index, serum PSA levels, smoking habitus, Gleason Scores, family histories,

Table I. *Demographic characteristics of the study population.*

Parameter	Prostate cancer (n=69)	Control (n=76)	*p*-Value
Age (years), mean±SD	67.61±7.349	67.53±8.771	0.967
Body mass index (kg/m2), mean±SD	27.01±3.715	27.29±3554	0.773
Smoking (pack years), mean±SD	30.56±18.686	27.75±17.043	0.594
PSA (ng/ml), mean±SD	48.339±103.377	3.036±2.669	0.001*
Family history of cancer, n (%)			
Yes	31 (44.9%)	-	-
No	38 (54.9%)	-	-
Gleason score, mean±SD	7.74±0.885	-	-

PSA, Prostate-spesific antigen; n, number of individuals; SD, standart deviation; Asterisk indicate statistically significant difference.

Table II. *Caspase 9 (CASP9) genotypic and allelic frequencies in ptostate cancer and control group.*

	Prostate cancer (n=69)	Control (n=76)	*p*-Value	Odds ratio	95% Confidence interval
Genotype	n (%)	n (%)			
CC	16 (23.2%)	16 (21.1%)	0.757	1.132	0.516-2.483
CT	38 (55.1%)	37 (48.7%)	0.442	1.292	0.672-2.484
TT	15 (21.7%)	23 (30.3%)	0.244	0.640	0.302-1.359
Allele	Allelic count n (%)	Allelic count n (%)			
C	70 (50.7%)	69 (45.3%)	0.244	1.562	0.736-3.317
T	68 (49.3%)	83 (54.7%)	0.757	0.883	0.403-1.937

n, Number of individuals.

tumor stages and pathological stages) of patients and controls collected from hospital records. DNA extraction was performed by iPrep Purification Instrument (Invitrogen, Life Technologies, Carlsbad, California, USA) by using 350 μl of whole blood and Invitrogen iPrep PureLink gDNA blood isolation kit (Invitrogen, Life Technologies, Carlsbad, California, USA). Isolated DNA samples were mesured spectrophotometrically with NanoDrop 2000 (Thermoscientific, Waltham, Massachusetts, USA), optical density ratios (at 260/280 nm) (mean=1.9±0.1) for genotyping and final concentrations of samples were diluted aproximately 100 ng/ μl. Genotyping for CASP9 gene rs1052576 polymorphism was performed by Applied Biosystems 7500 Fast Real-Time PCR instrument (Applied Biosystems, Foster City, CA, USA) by using TaqMan Genotyping Assay, TaqMan Genotyping Master Mix (TaqMan Reagents, Applied Biosystems, Foster City, CA, USA) and 100ng of sample DNA. Reaction mixture and conditions were used as recommended by manifacturer, mixture was containing 0.5 μl of TaqMan Genotyping Assay, 10 μl of TaqMan Genotyping Master Mix, 10 μl PCR grade water and 1 μl of sample DNA per well. The reaction conditions were 10 minutes at 95˚C Hold stage and 40 cycles of 15 sec. at 92˚C Denaturation and 60 sec. at 60˚C Annealing/Extention. Allelic discrimination of samples by collecting and interpreting flourescent signals of hybridization probes by software of 7500 Fast Real-Time PCR instrument. Statistical analyses were performed using SPSS Ver. 23 software (SPSS Inc, Chicago, IL, USA). The significancant differance between groups have examined by Student's *t*-test and demographic informations compared by Chi square and Fisher's exact tests. Risk estimations examined with Binary Logistic Regression analysis as odds ratio (OR) at 95% confidence interval (CI). $p<0.05$ denoted as statistically significant.

Results

The demographic characteristic of control and patient groups are given in Table I. The mean age of patients with prostate cancer and healthy controls was 67.61±7.34 and 67.53±8.77 years, respectively. No significant differences were found between patients and controls in terms of median age ($p=0.967$). PSA levels of patient group was 48.33±103.37 ng/ml and control group was 3.03±2.66 ng/dl. The patient group had significantly higher PSA levels ($p=0.001$) compared to the control group.

The allelic and genotypic frequencies for CASP9 (rs1052576) in patients with prostate cancer and controls are given Table II. There were no significant differences between the groups in the frequency of CASP9 genotypes ($\chi^2=1.363$; $p=0.506$). The frequencies of CC, TT, and CT genotypes among the patients with prostate cancer were 23.2%, 55.1% and 21.7%, respectively, and among the control subjects were 21.1%, 48.7%, and 30.3%, respectively. The frequency of CASP9(rs1052576) wild-type C allele was 50.7% in patient group and 45.3% in control group and there was no significant differences between the groups ($\chi^2=1.359$; $p=0.244$). Mutant

Table III. *Caspase 9 (CASP9) genotypic and allelic frequencies in clinical and pathological stages.*

	Clinical Stages n (%)			Pathological Stages n (%)				
	cT1c n=27 39.1%	cT2 n=34 49.3%	cT3 n=8 11.6%	T2a n=9 13%	T2b n=10 14.5%	T2c n=29 42.1%	T3a n=11 15.9%	T3b n=10 14.5%
Genotype		p=0.408				p=0.023*		
CC n=16 23.2%	n=7 43.8%	n=9 56.2%	n=0 0%	n=2 12.5%	n=4 25%	n=4 25%	n=4 25%	n=2 12.5%
CT n=38 55.1%	n=16 42.1%	n=17 44.7%	n=5 13.2%	n=1 2.4% ¥	n=5 13.2%	n=19 50%	n=6 15.8%	n=7 18.6%
TT n=15 21.7%	n=4 26.7%	n=8 53.3%	n=3 20%	n=6 40.1% ¥¥	n=1 6.6%	n=6 40.1%	n=1 6.6%	n=1 6.6%
Allelle								
C n=54 78.2%	n=23 42.6%	n=26 48.1%	n=5 9.3%	n=3 5.5% ¥¥¥	n=9 16.5%	n=23 46%	n=10 18.5%	n=9 16.5%
		p=0.369				p=0.010**		
T n=53 76.2%	n=20 37.7%	n=25 47.2%	n=8 15.1%	n=7 13.2%	n=6 11.3%	n=25 47.3%	n=7 13.2%	n=8 15%
		p=0.225				p=0.233		

n, Number of individuals.
*p=0.023, χ^2=23.540
**p=0.010, χ^2=16.937
¥p=0.004, χ^2=8.084, OR=0.078, 95% CI=0.0009-0.062).
¥¥p=0.000, χ^2=12.279, OR=11.333, 95% CI=2.39-53.748).
¥¥¥p=0.002, χ^2=12.279, OR=0.088, 95% CI=0.019-0.418).

T allele frequencies were 49.3% and 54,7% in patient and control groups, respectively (χ^2=0.096; p=0.757).

Distributions of CASP9 (rs1052576) genotypes according to clinical and pathological stages of prostate cancer were shown in the Table III. There was a significant difference between the groups by the pathological stages (χ^2=23.540; p=0.023). However, there were no significant differences between the patient and control groups in CASP9 (rs1052576) genotype frequencies by clinical tumor stage (p=0.408).

Patients with CASP9 (rs1052576) CT genotype was 12.8 fold higher in pathological stage of pT2a to any other stages of cancer (OR=0.078, 95% CI=0.009-0.062; p=0.004). In addition to that TT genotype carriers were 11.3-times lower in pathological stage of pT2a (OR=11.33, 95% CI=2.39-53.748; p=0.000). There was no significant difference between the groups for CC genotype (p=0.950). We also analyzed allele frequencies due to clinical stage and pathological stages of patients. There were no significant differences between clinical stages by CASP9 (rs1052576) C allele (p=0.369) and T allele (p=0.225). Nevertheless there was a statistically significant difference between the pathological stages and allele frequency (χ^2=16.937; p=0.010). Patients with C allele was 11.36 fold higher in pathological stage of pT2a to any other stages of cancer (OR=0.088, 95% CI=0.019-0.418; p=0.002).

Discussion

In this study, we aimed to analyze the relationship of CASP9 polymorphism and prostate cancer. We also analyzed the effects of CASP9 variations on the clinical charecteristics of prostate cancer pathology. Thus, gene and allele frequencies of *CASP9* (rs1052576) was determined based on clinical and pathological stages of patients.

Programmed cell death or apoptosis is an essential cellular defense mechanism against cancer development (4). There are two main signaling pathways: the extrinsic pathway and the intrinsic pathway each regulated by genes which identified in various human malignancies. The intrinsic pathway is controlled by members of the Bcl-2 family and initiates by the release of cytochrome *c* from mitochondria (4, 5). Then the release of cytochrome *c* from the intermembrane space of mitochondrion

activates CASP9 through the signal transduced by APAF1. Released cytochrome *c* interacts with APAF1, proCASP-9 and dATP to form an apoptosome. CASP9 is activated by the binding to the apoptosome, which subsequently triggers a cascade of effector caspases. The extrinsic pathway ligand pathway is initiated by the binding of death receptors to their corresponding extracellular ligands. The interactions between the ligands and membrane receptors activate the downstream death-inducing signaling complex, primarily composed of the Fas-associated death domain interacting with the death receptors through the death domains on both molecules (6).

Caspases are key regulators of apoptotic intrinsic or extrinsic pathways. Apoptosis is a prominent form of cell death characterized by a series of morphologic and biochemical changes, and is significance has emerged in numerous cancer types. It has been hypothesized that some CAP9 polymorphisms, particularly their haplotypes, can influence CASP9 expression, thus modulating susceptibility to cancer (7). Lin *et al.* (2012) showed that CASP9 may cause apoptosis inhibition and as a result of this effect this can cause susceptibility to lung cancer. They identified several CASP9 variants affecting the lung cancer susceptibility (8).

The molecular changes of different components of the apoptotic cascade have been identified in prostate cancers. Kesarwani *et al.* (2010) investigated the potential association between CASP9 polymorphism and the risk of prostate cancer. They showed that promoter polymorphism of CASP9 might influence activity of the gene in addition to its association with reduced risk of prostate cancer. CASP9 wild type allele may confer protection agaist prostate cancer (9). Recently, George *et al.* 2012 reported association of the CASP9 (rs1052576) polymorphism with tumor grade and bone metastasis. They recognized that CASP9 -1236 A>G was associated with decreased risk of occurrence of bone metastasis in patients with prostate cancer. These results suggested an important role of CASP9 in progression of prostate cancer rather than initiation (10).

In this study we found that *CASP9* (rs1052576) wild-type C allele was associated with decreased risk for pathological stage of prostate cancer patients. In context to our study, a meta-analysis of CASP9 polymorphism and prostate cancer susceptibility was performed by Xu *et al.*, 2012 (11). Their data showed that wild-type variation of *CASP9* gene contributed to decreased susceptibility to cancer in Caucasians and prostate cancer. The intrinsic initiator *CASP9* polymorphism may help to identify risk for the patients with prostate cancer. One important property of *CASP9* gene polymorphisms is that the incidence can differ substantially among different populations. Their data demonstrated significant differecences in the prevalence of the *CASP9* allele between Caucasion and Asians populations (11).

In conclusion, we showed that *CASP9* (rs1052576) C allele was related with a decreased risk for pathological stage of patients with prostate cancer. In addition to that, CT genotype was having positive impact on patholgical stage of patients with prostate cancer. *CASP9* (rs1052576) TT genotype was associated with higher risk of pathological stage. Those results implicated that *CASP9* variations could be associated with severity of prostate cancer pathology. Although genetic polymorphisms often show ethnic differences, further studies are needed in various populations to determine the realtionship between caspase gene polymorphisms and clinical characteristics of prostate cancer.

Conflicts of Interest

The Authors have declared that no conflict of interest exist.

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